



nanosString

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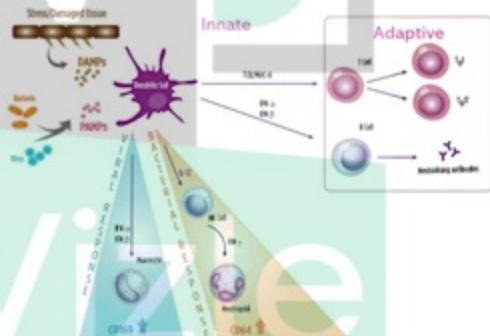
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Measuring the Immune Response with NanoString

- The immune response is key in cancer, infectious disease, autoimmunity, homeostasis, and so many other contexts
- T cells play a vital role in each of these, recognizing non-self antigens and eliminating threats
- The diversity of the T cell receptor (TCR) repertoire is a key readout of T cell activity



to autoimmune disease
to infectious disease to homeostasis.



Origin of TCR Diversity Using nCounter

Imaging, Diagnosis, Prognosis

Clinical
Cancer
Research

A New Approach to Simultaneously Quantify Both TCR α - and β -Chain Diversity after Adoptive Immunotherapy

Mingyue Zhang¹, Saunders Hall², Charlene Semperich², Helen Huo², Brian Rabinovitch², Richard E. Champer², Luis M. Varela², Patrick Hwu², Lucinda Reddy², T. and Laurence J.N. Cooper^{1,2}

Abstract

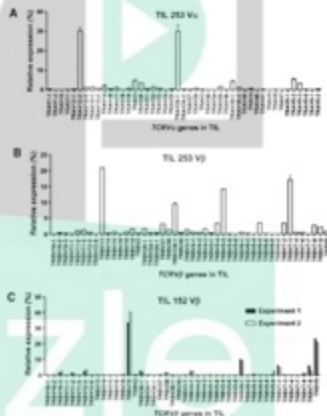
Purpose: T-cell receptor (TCR) variable α and β gene diversity is a surrogate biomarker for the therapeutic potential of adoptive immunotherapy and cellular immunity. Therefore, creating a sensitive, forward, rapid, sensitive, and reliable method to view the global changes of both TCR α and β transcripts in heterogeneous populations of T cells is appealing.

Experimental Design: We designed a "direct TCR expression assay" (DTREA) using a panel of customized bar-coded probes that simultaneously detects and quantifies TCR α and TCR β transcripts in a semiquantitative digital multiplexed assay from a small number of cells (10^3 cells) or as little as 100 ng of total RNA.

Results: We evaluated DTREA on total RNA samples of tumor-infiltrating lymphocytes and peripheral blood obtained from patients with melanoma after adoptive T-cell therapy. DTREA detected a unique spectrum of the distinctive patterns of TCR α gene usage in sequencing-based TCR α CDR3 regions. However, DTREA was rapid, achieved a level of sensitivity to identify rare T-cell populations, and simultaneously tracked the full array of α and β transcripts.

Conclusions: DTREA accurately and sensitively tracks changes in TCR α and TCR β gene usage in T-cell pools following immune interventions, such as adoptive T-cell transfer, and may be used to assess impact of modulation or reconstitution of T-cell compartment after hematopoietic stem cell transplantation. *Clin Cancer Res* 18(17):4253-62. ©2012 AACR.

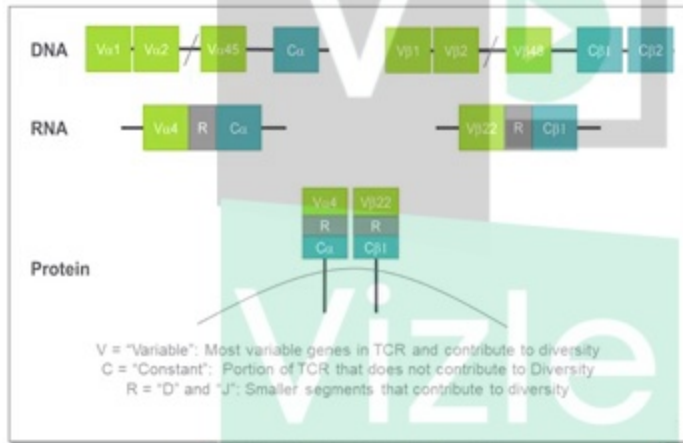
Example Data



with a group from MD
Anderson, the lab of Laurence Cooper,

nanoString

Simplified TCR Structure



Measuring TCR Diversity with nCounter

- NanoString measures which variable segment was chosen, rather than the full CDR3
- This approach is comparable to spectratyping, albeit the assay captures all variable segments of alpha, beta, gamma and delta TCRs
- The diversity of the TCR population is reported by the TCR diversity analysis module, and can be comparable to diversity indices reported by sequencing

NanoString



Sequencing

GAGGCATATTATACG

AGGTGCCAGTCCTTA

This is different from, say sequencing, where you might sequence



TCR Diversity: At a Glance

Chain Type	Constant Chains	Variable Chains
Alpha	TRAC	TRAV_ 45 probes, 46 genes TRAV8-2 and 8-4 covered by 1 probe
Beta	TRBC1/2	TRBV_ 46 probes, 48 genes TRBV6-3 and 6-2 covered by 1 probe TRBV12-4 and 12-3 covered by 1 probe
Gamma	TRGC1, TRGC2	TRGV_ 5 probes, 6 genes TRGV3 and 5 covered by 1 probe
Delta	TRDC	TRDV_ 3 probes, 3 genes
Immune Cell Markers		
CD3DIE/G, CD4, CD8A/B, PTPRC (CD45), CD45RO, CD45RA, SELL (CD62L), CCR7, CD28, CD40LG, IL2RA (CD25), NCR1 (NKp46)		

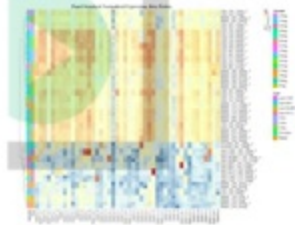
- Covers all alpha, beta, gamma, delta V and C segments
- Immune Cell Markers
 - Naive T cells
 - Memory T cells
 - Activated T cells
 - NK Cells
- Included in the CAR-T Characterization and TCR Diversity Panels



cells, as well as a few markers of NK cells to also determine

Analyzing TCR Diversity with nCounter

- Because nCounter has a unique way of summarizing TCR diversity, we created a report that reads out the results of the TCR Diversity Panel
- The analysis gives QC metrics, heatmaps, and finally a value called the "TCR Score", which is a Shannon Entropy metric to estimate TCR diversity
- Rosalind offers a cloud-based solution for this analysis, with answers for up to 96 samples in 10 min or less



and then, that TCR Score can be compared amongst groups or timepoints





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